PROBLEMS ASSOCIATED WITH REGISTERING HYBRID RAPE VARIETIES

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ABSTRACT

Hybrid varieties are becoming an established part of winter and spring rape cropping in the UK. However, their entry for trials for the National List and Plant Breeders' Rights has created a number of difficulties. Value for cultivation and use (VCU) testing of hybrids has been adapted to address a number of issues. Typically, commercial seed rates for hybrids are now 3.5 kg/ha, compared to 6–7 kg for non-hybrids. In the early years of testing, hybrids were grown at the same seed rates as non-hybrids (120 seeds/m^2) to allow direct comparison of yield and agronomic characters. However, experimental evidence suggested that this overestimated hybrid yields so their seed rates have been reduced to 70 seeds/m^2. Inter-plot competition between hybrids and conventional lines is also a subject of concern and trial designs have moved to blocking varieties by height. Additionally, semi-dwarf hybrids entering trials are grown in buffered plots to minimise the effects of substantial height differences. The testing of hybrid varietal associations (VA's) raised questions regarding the reliability of cross pollination. VA's are now grown as separate, buffered blocks, within the main trials to allow comparison with male fertile types.

Workloads associated with the identification and characterisation of hybrids for distinctness, uniformity and stability (DUS) purposes has increased three or four times for each entry because of the requirement to examine parental lines as well as the hybrid. New uniformity standards have been set to allow for the levels of hybrid purity associated with different hybrid systems. Considerable differences in DUS testing for hybrids exist across EU member states and harmonisation would be desirable.

HYBRIDS IN THE UK

Oilseed rape hybrids first entered trials for harvest 1993. A number of hybrid systems have been investigated and examples of some of these have been added to the UK National and Recommended Lists. Although up-take by growers has been cautious, it is estimated that hybrids represent 25% of the winter rape area and 30% of the spring area for harvest 1999. The following systems have been trialled in the UK:

- Hybrid varietal associations using: Ogu-INRA cytoplasmic male sterility
- Restored hybrids using: Self incompatibility
  Polima cytoplasmic male sterility
  Lembke male sterility
- Three-way crosses using: Ogu-INRA cytoplasmic male sterility
- Top crosses using: Ogu-INRA cytoplasmic male sterility
- GM herbicide tolerant hybrids using: Liberty-Link

The entry into trials of the different types is given in Tables 1 and 2.

Table 1 Winter oilseed rape: entries into official trials

Type		1993	1994	1995	1996	1997	1998	1999
Varietal associations	***Note:***	1	2	2	15	7	7	5
Restored hybrids	*entries appear*	-	-	4	2	2	13	18
Three-way hybrids	*in one*	-	-	-	-	3	1	8
GM hybrids	*category*	-	-	-	-	-	3	2
Top cross hybrids	*only*	-	-	-	-	-	1	-
Total entries (including line varieties)		46	47	48	60	62	63	71

Table 2 Spring oilseed rape: entries into official trials

Type		1993	1994	1995	1996	1997	1998	1999
Varietal associations	***Note:***	-	2	3	2	8	2	1
Restored hybrids	*entries appear*	1	1	4	2	-	1	3
Three-way hybrids	*in one*	-	-	-	-	-	1	-
GM hybrids	*category only*	-	-	2	2	1	2	4
Total entries (including line varieties)		28	19	30	20	27	25	21

PERFORMANCE TESTING

Testing hybrids in variety trials for their value for cultivation and use (VCU) as part of the official test protocol for National Listing has posed three principal problems:

- Seed rate for hybrids
- Pollination of varietal associations
- Inter-plot competition

Traditional seed rates for winter oilseed rape in the UK are in the order of 6.5-7.0 kg/ha (120 seeds/m^2), compared with as little as 2.0 kg in Germany and France. Comparatively high rates in the UK arise from difficult seedbed conditions (especially on clay soils) and pest problems in autumn. In the early years of hybrid testing in official trials, one protocol was used for hybrids and non-hybrids. This allowed direct comparison of yield and field characters. However, hybrids are marketed at much lower seed rates—typically 3.5 kg/ha. This is, in part, to reduce seed cost (hybrids are sold at £15-17/kg compared with £6-7/kg for non-hybrids). Sound agronomic reasons may also exist for growing hybrids at the lower rate because they are more vigorous and branching than conventional types. Lodging resistance benefits from lower plant density. In the case of varietal associations, there is also some concern that, at high populations, the pollinator component might be smothered.

Table 3 The effect of seed rate on seed yield of Synergy

Seed rate	Year			
	1996	1997	1998	Mean
60/70 seeds/m^2	103	103	111	106
120 seeds/m^2	107	107	114	109
Control yield (t/ha)	3.6	4.3	4.4	4.1

Seed rate was monitored in UK trials over a three year period using the varietal association Synergy. Table 3 lists the relative yield figures by seed rate showing a small but consistent yield advantage at the higher rate. The low rate was 60 seeds/m^2 in 1996 and 70 seeds/m^2 in 1997 and 1998.

Conversely, the apparent disadvantage of low seed rate in terms of reduced yield was off-set by improved straw characteristics, as illustrated by the mean data for lodging resistance and stem stiffness (Table 4). This is of critical importance to many growers, who prefer short, stiff varieties.

Table 4 Effect of seed rate on straw characteristics of Synergy (1-9 scale, 9 best)

Seed rate	Resistance to lodging				Stem stiffness			
	1996	1997	1998	Rating	1996	1997	1998	Rating
60/70 seeds/m^2	8.5	8.6	7.7	9	7.6	7.4	7.7	8
120 seeds/m^2	8.3	8.0	6.7	7	5.9	6.3	6.1	6

In order to take account of the commercial reality of lower seed rates for hybrids and the associated agronomic improvements, the seed rate has been changed to 70 seeds/m2 for all hybrid types in the official protocol.

Reliability of pollination

Pollination in varietal associations (VA's) has great significance for growers. Each VA is a mixture of male sterile hybrid plants (70-80%) with pollinator plants of one or more conventional varieties or hybrids making up the balance. In a crop normally considered to be highly self pollinating, the dependence on cross pollination for seed set has been a source of widespread concern. However, pollination of VA's is difficult to study and interpret. Attempts to assess yield and compare with yields of conventional varieties are invariably flawed:

- VA's grown in trials with fully fertile varieties are likely to benefit from an enhanced pollen supply, compared with that encountered in VA crops, with possible yield advantages.
- Yields of VA's grown in separate series, with a suitable isolation distance to avoid pollen flow from fully fertile types, cannot be related reliably to those of the fully fertile types.

As a compromise solution, UK trial protocols have been modified to introduce separate sub-blocks, bordered by a minimum of 6m of VA surround, to be randomised within conventional variety trials.

Inter-plot competition

Some breeders fear that the vigorous growth of some hybrids may have a significant detrimental influence on neighbouring plots. The EU pollination study has provided experimental evidence to support this (Kightley, 1998). Components of yield were investigated at isolated and non-isolated sites for Synergy at both seed rates. In addition, the conventional variety Falcon, the pollinator component of Synergy, was studied at the non-isolated sites. Thus, at the non-isolated sites, we were able to compare Falcon plants grown as a pure stand and in competition with hybrid plants at two seed rates (Table 5). As a useful indicator of plant development we compared the number of pods/plant at sites not isolated from male fertile rape.

Table 5 Pod numbers per plant: comparison of hybrid and pollinator plants. Mean data from three locations in 1997

Synergy				Falcon
Male sterile hybrid component		Pollinator component (Falcon)		as pure variety
70 seeds/m^2	120 seeds/m^2	70 seeds/m^2	120 seeds/m^2	120 seeds/m^2
399	280	181	140	202

The data show that: a) Falcon plants produced fewer pod than the sterile hybrid.
b) Falcon grown as a pure stand produced more pods than Falcon grown within a VA, even at the same overall population.

This evidence suggests that some hybrids show significantly greater competitive vigour than non-hybrid lines. We consider, however, that in plots with 60cm inter-plot gaps and a total width of more than two meters, competition effects are minimised. However, a MAFF-funded study of inter-plot competition is currently underway to confirm this.

As an interim measure trial layouts have been modified to use a 'neighbour restricted' design (David *et al*. 1996) to prevent varieties with extreme height differences being sown in adjacent plots.

PLANT BREEDERS' RIGHTS

For a new variety to be granted Plant Breeders Rights within the UK and the European Union it must be distinct, uniform, stable (DUS) and novel. These criteria are defined in broad terms in the 1991 UPOV convention and detailed technical protocols. In the UK, legal status is granted by the 1997 Plant Varieties Act (MAFF) which incorporates the UPOV convention. Commercialisation before testing normally disqualifies a candidate variety because it is no longer novel.

Testing hybrid oilseed rapes, particularly those using male sterility and multiple crosses in their derivation, presents major administrative and technical problems for DUS tests. DUS tests are normally carried out in field grown plots and the procedures used for hybrids should have commonality with those used for conventional varieties, they should also reflect resource constraints and set achievable standards without inhibiting plant breeders producing new and improved cultivars. It is necessary to harmonise approaches within EU member states and reach agreement with the European Community Plant Variety Rights Office (CPVO).The following UPOV principles also had to be maintained:

1. Distinctness should be based on phenotype.
2. Characters used to define the variety must be capable of precise recognition and description.
3. The new variety must exhibit sufficient uniformity consistent with the breeding system of the species; any variation must permit accurate description and assessment of DUS.

To meet these constraints, the following proposed procedures are currently being considered by the authorities for adoption in the UK after consultation of the industry:

- All hybrids to be declared *per se* distinct from non hybrids. Thus, UPOV terminology allows hybridity to be considered a distinguishing character (UPOV 1996).
- If the hybrid is not distinct using routinely recorded and computed characters at the required levels of significance the parental formula is used.

- To be distinct using the parent formula:
    1. There must be *some* evidence that the two non-distinct hybrid varieties are different, albeit not significantly different, in characters which are an expression of the genotype;
    2. At least one parent in the formula of one of the non-distinct hybrids must be distinct from both parents of the other hybrid.
- In hybrids involving more than a single cross, where a parent is a hybrid, it may be necessary to distinguish the grandparent lines.
- Experience has shown that 5-10% of the submitted lines are incorrectly labelled by the applicant for registration; before testing by formula it may be necessary to check by isozyme analysis that the parent lines supplied are consistent with the hybrid.
- Hybrids having hybrids as parents to be considered sufficiently uniform if segregation for specific characters such as male sterility is consistent with the genetic background and behaves in a predictable manner.
- For components produced by genetic modification, for example, genetic male sterility linked to herbicide resistance, PBR should be awarded to the untransformed female parent which confers protection to the transformed version because the latter cannot be produced without the former.

Experience may permit DUS to be established for hybrids using modified statistical criteria, multivariate statistical tests, use of data from performance trials and field recordings in side-by-side test plots by crop experts. At present there are no plans to use molecular techniques.

DUS methods have been modified to ensure that hybrids can be tested in an effective manner irrespective of the breeding system used.

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